A STUDY OF THE GONOCOCCUS AND GONOCOCCAL INFECTIONS*

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The purpose of this work was: first, to devise improved methods of diagnosis of gonorrhea, and, second, to determine whether typing of strains of gonococci could be made. The need of improved methods of diagnosis of gonococcal infections is well known. The advantages to be derived from a typing of the gonococcus, aside from a theoretical interest, lie in the possibilities thus offered of a better specific therapy. The methods of diagnosis which have been studied are both cultural and serologic. Attempts were made to develop a medium on which the gonococcus would grow even if present in small numbers and accompanied by contaminating organisms. This necessitated a study of the factors essential to growth, namely, nutrient substances, and certain conditions of the environment. Diagnosis by means of serologic procedures has included work on the alexin fixation reaction. The intracutaneous test was also studied. Typing of strains of the gonococcus was attempted by the usual fixation and agglutination tests, and also by the method of absorption of agglutinins.

Growth of Pure Cultures of the Gonococcus.—Cultural work was first concerned with the growing of stock cultures. The stock cultures consisted of 6 strains obtained from the Cutter laboratories and 10 strains which we had isolated, all of which had been obtained from acute cases of anterior urethritis in men. A number of mediums, which have recently been claimed to possess distinct advantages, were compared from the standpoint of growth favoring properties. In every instance the method of preparing the mediums as described by the authors has been faithfully followed. The solid mediums in the series were used as slants. The results are given in table 1.

As is evident, of mediums employed, testicular agar or testicular agar containing blood or hydrocele fluid proved the most satisfactory. In keeping stock cultures, testicular agar alone gave good

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results. For the invigoration of poorly growing strains, chocolate blood testicular agar was particularly advantageous. Alternation from testicular agar to chocolate blood testicular agar and back again for several generations invariably restored the growth of a weak strain. Testicular agar containing yeast possessed no advantages in growth producing properties over testicular agar alone, in spite of the fact that a vitamine factor may have been supplied. Eberson's semisolid yeast agar, which was advocated because of the property of maintaining viability in the case of meningococcus, gave no growth. Starch agar and tryptamine agar were disappointing. No growth was obtained in any fluid medium. The vigor of stock cultures was maintained most satisfactorily by transplanting at 3-day intervals and keeping at 37 C. Cultures left at room temperature or in the icebox were less active than those kept at incubator temperature.

TABLE 1

GROWTH FAVORING PROPERTIES OF VARIOUS MEDIUMS

Medium	Growth
Beef infusion agar	None
Starch agar (Vedder [1])	Poor
Tryptamine agar (Cole and Lloyd [2])	Fair
Beef infusion agar with 10% hydrocele fluid	Fair
Beef infusion agar with 10% blood	Good
Testicular agar (Hall [3]) (Clark [4])	Luxuriant
Testicular agar containing 10% hydrocele fluid	Luxuriant
Testicular agar containing 10% blood	Luxuriant
Testicular agar containing 10% blood coagulated (Chocolate blood agar)	Luxuriant
Testicular agar containing infusion of yeast	Luxuriant
Semisolid yeast agar (Eberson [5])	None
Beef infusion broth	None
Beef infusion broth containing 10% hydrocele fluid	None
Beef infusion broth containing 10% blood	None
Brain medium (von Hibler [6])	Very scanty
Ground testicular medium prepared like brain medium	Very scanty

The period of viability of gonococcus was determined with several mediums which were selected because of the results obtained in the study of their growth favoring properties. Testicular agar and chocolate testicular agar were chosen because of the active growth obtained. Yeast testicular agar was used because of the possibility that the vitamines supplied by the yeast would prove a factor favorable to prolonged growth. Ground testicular medium was also tried, as it offered conditions of environment similar to the brain medium on which

[1] Jour. Infect. Dis., 1915, 16, p. 385.
[2] Jour. Path. & Bacteriol., 1917, 21, p. 267.
[3] Jour. Bacteriol., 1916, 1, p. 343.
[4] Ibid., 1920, 5, p. 99.
[5] Abstr. Bacteriol., 1919, 3, p. 10.
[6] Untersuchungen über pathogene Anaeroben, 1908, p. 85.

Bradley [7] reported successful results with the meningococcus. The agar mediums were used in the form of slants, as preliminary experiments on stab cultures of these mediums showed no recoverable growth after only 3 days' incubation. Determinations on each medium were made in quadruplicate, as 4 strains were used for inoculation, but as the results with all 4 were uniform, they are not given separately. All tubes were held at 37 C. during the entire period of the test and subcultures made on the most favorable medium, chocolate testicular agar, every 2 days. The results are given in table 2.

TABLE 2

RESULT OF TESTS

Medium	Period of Viability at 37 C.
Testicular agar	8 days
Chocolate blood testicular agar	8 days
Yeast testicular agar	8 days
Ground testicular medium	8–12 days

The apparently slight advantage of ground testicular medium over the other mediums was offset by the fact that only scanty growth could be obtained on transplants from this medium. None of the mediums gave promising results from the point of view of prolonging the period of viability.

The effect of temperature on viability was determined with cultures on testicular agar. Cultures were grown for 48 hours at 37 C. and were then placed in the icebox at 4 C., at room temperature at about 20 C., and in the incubator at 37 C. Cultures from each series were viable 8 days and no longer. During the period of test, transplants from tubes kept at 37 C. showed somewhat more luxuriant growth than those of the room temperature and icebox series. However, the actual period of viability was no longer in the series kept at 37 C.

The environmental factors that have been considered of most importance in the growth of the gonococcus are moisture, the physical state of the medium and oxygen tension. Moisture in the atmosphere is generally accepted as a requirement for luxuriant growth. In the present investigation cultures were grown continuously in closed jars containing water. The growth in these jars was uniformly good. On the other hand, cultures kept in the open chamber of the incubator, even though a pan of water was always kept in the same chamber,

[7] Jour. Am. Med. Assn., 1918, 70, p. 1816.

never showed as early or as active growth as those in the closed jars. Saturation of the atmosphere with moisture seems therefore to be an essential factor in growth of the gonococcus.

The advisability of using moist or dry medium is a question on which there is disagreement. McCann,[8] Van Saun,[9] and more recently Jenkins [10] recommend a medium of high water content. Cole and Lloyd [2] consider a moist medium essential, but at the same time allow a certain period of maturation of the medium, during which time they claim that there occurs a concentration of adsorbable hormones at the surface. Hall [3] advises the use of a heavy agar free from excessive moisture. Our work has shown that maximum growth is obtained on a medium containing 2.5% agar from which excessive moisture is removed by 24 hours' incubation at 37 C. and 24 to 48 hours at room temperature. The character of the surface of the medium was found to be an important factor. A hard smooth surface produces good growth, while cultures grow poorly on agar which is soft and easily broken. Water of condensation is not favorable to growth.

The value of oxygen tension as an environmental factor is again a disputed question. Wherry and Oliver,[11] Ruediger,[12] Swartz, Shohl, and Davis [13] all find a reduced oxygen tension favorable to growth of the gonococcus. Chapin [14] obtains better results with an atmosphere in which 10% of air is replaced by carbon dioxide than in ordinary atmosphere. Herrold [15] and Hermanies [16] advocate the growing of cultures in closed systems in the presence of cultures of B. subtilis. They find this procedure of particular value for the isolation of cultures. In the present work, the value of reduced oxygen tension was tried by running 2 series of parallel cultures—one grown in a moist atmosphere in which 10% of the air was replaced by carbon dioxide, the other in an identical moist atmosphere without carbon dioxide. No differences were observed in the two series with respect to the abundance or rapidity of growth. Stock cultures and newly isolated cultures were grown in closed systems with cultures of B. subtilis. No improvement

[8] Lancet, 1896, 1, p. 149.
[9] Dept. of Health, N. Y., 1913, 7, p. 101.
[10] Jour. Path. & Bacteriol., 1921, 24, p. 160.
[11] Jour. Infect. Dis., 1916, 19, p. 288.
[12] Ibid., 1919, 24, p. 376.
[13] Johns Hopkins Hosp. Bull., 1920, 31, p. 449.
[14] Jour. Infect. Dis., 1918, 23, p. 342.
[15] Jour. Am. Med. Assn., 1921, 76, p. 225.
[16] Infect. Dis., 1921, 28, p. 133.

in growth was noted over that obtained in a moist atmosphere. We must conclude therefore that the value of a reduced oxygen tension has been overestimated.

Isolation of Cultures of Gonococcus.—Isolation of cultures was attempted from two types of cases—from acute cases of anterior urethritis in men and from chronic cases in women. The procedure varied according to the type of case.

In acute anterior urethritis in the male, after cleansing the meatus and expressing several drops of pus, cultures were made directly on slants of chocolate blood testicular agar. If the discharge was copious, pure cultures were sometimes obtained directly. Even when contaminating organisms were present in fairly large numbers, a sufficient growth of gonococcus was usually obtained to permit a later isolation. The gonococcus was isolated from 50% of 20 untreated cases of acute anterior urethritis in the male. These cultures were kept on chocolate blood testicular agar for the first 8 or 10 generations, after which they could be transferred to testicular agar.

In isolation of cultures from the cervix uteri in chronic cases of gonorrhea in women, measures were required to inhibit the growth of contaminating organisms. The studies of Churchman,[17] Browning and Gilmour,[18] Drennan and Teague,[19] Dreyer, Kriegler and Walker,[20] Krumweide and Pratt,[21] Gay and Morrison[22] have demonstrated the selective inhibitory action of certain dyes of the triphenylmethane series for a number of organisms. Gentian violet inhibits the growth of staphylococcus and other gram-positive organisms in dilutions which have no effect on gram-negative forms. The inhibitory action of brilliant green for B. coli is known. Brilliant green, malachite green, methyl violet and solid green are distinctly bactericidal for streptococcus and to a less extent for staphylococcus. On the basis of these facts, it seemed possible that a dye or combination of dyes might be found which would inhibit the growth of the vaginal flora, at the same time permitting the growth of the gonococcus. Accordingly, a number of dyes were selected for a comparative study of their inhibitory action on the gonococcus, staphylococcus, streptococcus and B. coli. These were gentian violet, methyl violet, brilliant green, solid green, malachite

[17] Jour. Exper. Med., 1912, 16, p. 221; 17, p. 373.
[18] Jour. Path. & Bacteriol., 1914, 18, p. 144.
[19] Jour. Med. Res., 1916, 35, p. 519.
[20] Jour. Path. & Bacteriol., 1911, 15, p. 133.
[21] Jour. Exper. Med., 1913, 29, p. 20.
[22] Jour. Infect. Dis., 1921, 28, p. 1.

green, basic fuchsin, pyronin and Spiller's purple. The dyes of the acridine group, although distinctly active against streptococcus and staphylococcus, were not included in the series, as members of this series are known to possess a marked inhibitory action for the gonococcus.

The effect of the dyes was first determined on stock cultures of the organisms in question. Inoculation of cultures was made on testicular or chocolate blood testicular agar to which had been added dye in different concentrations. The P_H of the medium was 7.6. The action of the dye was shown by the amount of growth obtained after 48 hours' incubation. The results are given in table 3.

TABLE 3

INHIBITORY ACTION OF DYES ON VARIOUS ORGANISMS

Medium	Dye	Dilution	Gonococcus	Streptococcus	Staphylococcus	B. coli
Testicular agar	Gentian violet	1:50,000	+ −	−	+ −	+ +
		1:100,000	+	−	+	+ +
		1:500,000	+ +	−	+	+ +
Chocolate blood testicular agar	Gentian violet	1:50,000	+ −	−	+	+ +
		1:100,000	+ +	−	+	+ +
Testicular agar	Methyl violet	1:100,000	+	+ +	+ −	+ +
		1:500,000	+ +	+ +	+	+ +
Testicular agar	Brilliant green	1:250,000	+ −	+	+ +	+ +
Testicular agar	Solid green	1:100,000	+	−	+	+ +
		1:500,000	+ +	+ −	+ +	+ +
Testicular agar	Malachite green	1:100,000	+ −	−	+ −	+ +
		1:500,000	+ +	−	+ −	+ +
Testicular agar	Basic fuchsin	1:100,000	+ −	−	−	+ +
		1:500,000	+	−	+ +	+ +
Testicular agar	Pyronin	1:100,000	+	+ +	+ +	+ +
		1:500,000	+	+ +	+ +	+ +
Testicular agar	Spiller's purple	1:100,000	+ −	+ +	+ +	+ +

It is evident that of the dyes tested none fulfil the requirements which would give ideal conditions for the isolation of the gonococcus. In certain cases there is a retardation, if not complete inhibition of streptococcus and staphylococcus, but no dye inhibits B. coli in a concentration which permits growth of the gonococcus. Gentian violet, solid green, and malachite green have no inhibitory action on the gonococcus in a dilution of 1 : 500,000. Staphylococcus is partially inhibited by solid green and malachite green. As the gram-positive cocci seem to be the most difficult of separation from the gonococcus in primary cultures, mediums were prepared containing separately gentian violet, malachite green, and solid green.

The nutrient base of the mediums used in isolation was testicular agar. To this was added in some cases 10% blood, which was coagulated to make chocolate blood agar, in other cases 10% hydrocele fluid. It was found that the hydrocele agar was the more satisfactory, since the chocolate blood medium was too opaque to permit the fishing of single colonies. For isolation plates were used. Cultures were taken from within the cervix; pus was smeared over the surface of the medium and the plates were incubated immediately. Plates were examined after 2, 3, and 4 day incubation. A known culture of gonococcus was planted on each series of plates to control the growth producing properties of the medium. Good growth was obtained with this control culture on every medium. The results of cultures made on various mediums will be given.

Chocolate Blood Testicular Agar Containing Gentian Violet 1:500,000.—Cultures were taken in twenty-nine cases. Smears for microscopic examination made at the time cultures were made showed only one case in which intracellular gram-negative diplococci were demonstrated. Five showed many extracellular gram-negative diplococci. All were considered clinically chronic cases of gonorrhea. In 10 cultures of this series, the 48-hour growth gave colonies which on microscopic examination were gram-negative diplococci, morphologically typical of gonococcus. Fishings made from these 10 plates on chocolate blood testicular agar gave in 3 cases growths which appeared to be cultures of gram-negative diplococci. We were unable to keep these cultures after 3 or 4 generations, however. In many cases, colonies which were typically gram-negative in their staining properties when fished from the plates containing gentian violet gradually developed a gram-positive character with successive transplants on chocolate blood testicular agar containing no gentian violet. As no pure cultures were isolated from this series, we cannot say that the morphologically typical organisms occurring on the primary cultures were gonococci. However, in this and succeeding series, the growth of biscuit shaped gram-negative diplococci from a gonorrheal discharge was considered at least suggestive of gonococcus.

Chocolate Blood Testicular Agar Containing Gentian Violet 1:500,000 and Soap 1:2,000 (Loco Castile Soap).—This medium was prepared like the other except that a solution of soap was added in the hope of approximating conditions obtained by Avery[23] with the

[23] Jour. Am. Med. Assn., 1918, 71, p. 2050.

influenza bacillus. Of 16 cultures, none gave positive microscopic evidence of gonococcus. Colonies of gram-negative diplococci were obtained in the primary cultures from 4 cases but no pure cultures were isolated.

Chocolate Blood Testicular Agar; No Dye or Soap.—As controls on the two series mentioned in the previous paragraph, cultures were made on chocolate blood testicular agar with no dye or soap, of 6 cases negative according to microscopic examination. Colonies of gram-negative diplococci were obtained in primary culture from 3 cases. No pure cultures were isolated.

Hydrocele Testicular Agar Containing Gentian Violet 1: 500,000.—Of 30 cultures, 4 were positive according to microscopic examination made at the time of cultivation. In 8 of these cases gram-negative diplococci were obtained on the primary culture. No pure cultures were isolated.

Hydrocele Testicular Agar Containing Solid Green 1: 500,000.—Cultures were made from 8 cases. None was positive on microscopic examination. One gave gram-negative diplococci in primary culture. No pure cultures were isolated.

Hydrocele Testicular Agar Containing Solid Green 1: 500,000; Suspension of Yeast 1: 1000.—A suspension of yeast was added in the hope of encouraging the growth of the gonococcus by supplying vitamines. The results were no different from those without yeast.

Hydrocele Testicular Agar Containing Malachite Green 1: 500,000.—Cultures were made from 2 cases. No gram-negative diplococci were obtained.

Hydrocele Testicular Agar, No Dye or Yeast.—Parallel plates containing no dye were inoculated from the same cases as those of the before-mentioned series. Gram-negative diplococci appeared in a slightly lower percentage of plates. No pure cultures were obtained. Contaminating organisms were present in somewhat greater numbers and appeared earlier than in plates containing dye.

While the results show that the presence of dyes in the mediums inhibited the growth of contaminating organisms to a slight extent, the inhibition was nevertheless not sufficient to permit the isolation of the gonococcus. However, the conditions of the experiment were difficult, since of 91 specimens taken, only 5 were positive on microscopic examination. With the exception of these 5 cases, it is impossible to say definitely whether the failure to obtain pure cultures was

due to the fact that the organisms were not present in the discharge or whether sufficiently favorable conditions of growth were not provided. However, the fact that pure cultures were not obtained from the 5 cases in which gonococci were demonstrated microscopically would indicate that the conditions of growth were not adequate for the gonococcus. Moreover, the appearance of colonies of typical gram-negative diplococci in primary cultures, subcultures of which were impossible to grow, is also indicative of the need of perfecting the medium before isolation from chronic cases may be accomplished. The results obtained from the foregoing series, namely, the greater percentage of gonococcus-like colonies and the lower percentage of contaminating organisms on plates containing dye, could indicate that the possibility of successful isolation of the gonococcus lies in the perfection of a selective medium.

IMMUNITY TESTS IN GONOCOCCAL INFECTIONS

The Alexin Fixation Test in Gonorrhea.—Before attempting to determine the value of the alexin fixation test in the diagnosis of gonorrhea, a number of antigens were prepared and tested for their efficiency. All of these antigens were polyvalent, representing in every case growth from eight strains.

The first series of antigens were suspensions of 48-hour growth of gonococcus in normal salt solution. These were allowed to autolyze at different temperatures by shaking at room temperature for from 6 to 8 hours with subsequent standing in the icebox for 48 hours, by allowing to remain in the incubator for 48 hours, and by heating to 56 C. for 2 hours. Five-tenths per cent. phenol was added to all of the suspensions. After autolysis, tests were made on the inhibitory and antigenic properties of each whole preparation and also on the supernatant fluid obtained by centrifugalization of a portion of each with an immune serum. No appreciable differences were observed in any of these antigens. Neither did the supernatant fluid differ from the whole suspensions. In every case, 2 antigenic units equaled from ¼ to ⅕ the inhibitory dose.

Other antigens prepared and tested were: a suspension of organisms subjected to N/10 NaOH one-half hour with subsequent neutralization with N/1HCl; organisms extracted with alcohol and ether according to the method recommended by Smith and Wilson [24]; an antigen dialyzed according to the method recommended by Wadsworth and

[24] Jour. Immunol., 1920, 5, p. 499.

Maltaner [25] for use with B. tuberculosis. Of these 3, the first 2 were relatively nonantigenic, while the third was slightly more inhibitory to alexin than the antigens of the first series. Therefore, in all fixation tests used for the purpose of diagnosis, the antigens used was a polyvalent suspension of organisms in normal salt solution, phenolated and autolyzed at 56 C.

Fixation tests of serum of gonorrheal patients were made using the antisheep hemolytic system, 2 units of alexin, fixation at 37 C. for one hour, with further incubation of one-half hour after the addition of sensitized cells. Eighty serums were tested. These are classified and the results given in table 4. A + + reaction represents complete inhibition.

TABLE 4
RESULTS OF FIXATION TESTS

Type of Serum	Total Number	Positive	Negative
Gonorrhea:			
Early acute	4	2 + —	2
Cured acute 8 weeks after onset	2	2 + +	0
Chronic	9	4 + + 2 +	3
Arthritis	1	1 + +	0
Posterior urethritis	1	1 + +	0
Doubtful diagnosis	2	0	2
Tuberculosis	16	1 + +	15
Syphilis	16	0	16
Respiratory, skin and other diseases	20	1 + —	19
Normal	9	0	9

It is difficult to draw conclusions on the efficiency of a test when so small a series of cases is represented. Nevertheless, certain facts are evident. Early acute cases gave negative or slightly positive reactions. Chronic cases, including gonorrheal arthritis, were positive in 72.8%. Serums taken immediately after cure was established clinically were positive. Normal serum and serum from diseases other than gonorrhea were negative, with the exception of a + + reaction in one tuberculosis patient. No serum from cases of meningitis was available. The cross reaction so commonly observed between gonococcus and meningococcus could therefore not be tested with meningitis serum. A number of + + gonorrheal serums, however, were tested against a meningococcus antigen and were found to give negative or at the most slightly positive reactions.

These results corroborate those of other investigators, among whom may be mentioned Teague and Torrey,[26] Watabiki,[27] Kolmer and

[25] Jour. Exper. Med., 1921, 33, p. 119.
[26] Jour. Med. Res., 1907, 17, p. 223.
[27] Jour. Infect. Dis., 1910, 7, p. 159.

Brown,[28] Irons and Nicoll,[29] and Smith and Wilson.[24] The fixation test must be considered a valuable aid in the diagnosis of all except the early acute cases of gonorrhea.

Agglutination and precipitin tests were not tried in diagnosis, since these tests gave less distinctly positive results with known antigonococcus immune serum than did the fixation reaction.

The Intracutaneous Reaction in Gonorrhea.—Bruck,[30] Köhler,[31] and Irons [32] studied the reaction of gonorrheal patients to the cutaneous and intracutaneous application of preparations of gonococcus. While their studies indicate that these tests are of diagnostic value, the results were not found entirely reliable, especially in certain types of gonorrhea. Moreover, according to the work of Irons, positive reactions often occur in gonorrheal patients on the application of a preparation of meningococcus. A cross reaction was also obtained with gonococci in meningitis patients. It seemed possible that an improvement might be made in this test by using a different preparation of antigen. The results of Gay and Force [33] with typhoidin and of Gay and Minaker [34] with a preparation of meningococcus suggested the use of a similar antigen in gonorrhea. It was hoped that this antigen would prove more active and more specific than the preparations used by Bruck, Köhler and Irons.

The technic of preparation of this antigen was as follows: Seventy-two hour cultures of 8 strains of gonococcus on testicular agar were washed off in water. The aqueous suspension was placed in the incubator for 48 hours to allow autolysis of the organisms. From 10 to 15 volumes of 95% alcohol were then added. The mixture was shaken and allowed to stand for 24 hours. The supernatant fluid was removed and the precipitate washed once in 95% alcohol and twice in absolute alcohol. The precipitate was then shaken with ether, the ethereal suspension poured on hard filter paper and washed with absolute ether. The residue was dried over sulphuric acid at 40 C. When apparently dry, the product was ground to a powder in an agate mortar and kept in a desicator at 40 C. until of constant weight. For use this powder was suspended in phenolated salt solution. The

[28] Ibid., 1914, 15, p. 6.
[29] Ibid., 1915, 16, p. 303.
[30] Deutsch. med. Wchnschr., 1909, 35, p. 470.
[31] Wien. klin. Wchnschr., 1911, 24, p. 1564.
[32] Jour. Infect. Dis., 1912, 11, p. 77.
[33] Arch. Int. Med., 1914, 13, p. 471.
[34] Jour. Am. Med. Assn., 1918, 70, p. 215.

optimum concentration was found to be such that 0.05 c c contained 0.0066 mg. of dried powder. As a control a similar preparation of meningococcus was made.

Intracutaneous injections of 0.05 c c of this suspension were given to a series of patients with gonorrhea and a series of normal individuals. A white wheal was the immediate result of injection. The reaction was considered positive if 48 hours after injection there was a marked area of erythema and distinct induration. A slight erythema was probably due to the toxicity of the preparation and could not be considered as a positive reaction. The results are given in table 5.

TABLE 5

RESULTS OF INJECTIONS WITH GONOCOCCIN AND MENINGOCOCCIN

Type of Case	Number	After Injection with Gonococcin			After Injection with Meningococcin		
		Erythema Induration	Slight Erythema	No Reaction	Erythema Induration	Slight Erythema	No Reaction
Acute epididymitis	1	0	0	1	0	0	1
Chronic double epididymitis	1	1	0	0	1	0	0
Prostatitis	3	1	2	0	2	1	0
Arthritis	1	0	1	0	0	1	0
Acute anterior urethritis 1 week cured	1	1	0	0	1	0	0
Anterior urethritis 3 mos. and 2 years	4	0	1	3	0	1	3
Normals	18	1	1	16	2	1	15

From the small series of cases tested, gonococcin cannot be considered to constitute an aid in diagnosis. Three cases of chronic gonorrhea gave positive reactions with gonococcin, but three equally strong positive reactions were obtained with meningococcin. In general, the reactions with meningococcin were identical with those with gonococcin both in gonorrheal cases and in normal cases. In view of these facts, no further work was done with the intracutaneous reaction.

Typing of Strains of Gonococci.—Sixteen strains of gonococcus were used in the work on typing. A strain of meningococcus was also included in this study as a control. The strains of gonococcus exhibited no cultural differences. Their fermentative reactions were also identical—all strains giving slight acidity on a dextrose agar with Andrade's indicator. The procedures by means of which typing was attempted were the alexin fixation and agglutination reactions and also absorption of agglutinins of immune serums.

Immune serums were obtained by injection into rabbits intraperitoneally with washed cultures of individual strains. Washed cultures were found to be much less toxic than suspensions of growth which had not been washed. The dosage was gradually increased from one-half culture to 8 to 10 cultures on testicular agar slants. Fifteen to 20 injections were given at intervals of from 3 to 5 days. The production of an immune serum was difficult as the injections were in many cases followed by loss of weight and death of the animals.

Fixation reactions were made with all serums against antigens prepared from each individual strain. Antigens were prepared according to the procedure adopted in the fixation tests reported in the work on diagnosis, except that, of course, in these cases each antigen comprised only one strain. A serum was used for classification only when its fixation titer with the specific strain was at least 1 : 400.

The results of fixation of each serum with its specific strain and with the heterologous strains showed no characteristic or consistent differences in titers with different antigens. Of 8 serums produced by immunizing against individual strains, 3 gave slightly stronger reactions with the specific than with the heterologous antigens, while 5 gave stronger reactions with heterologous antigens. All serums gave positive reactions with all the gonococcus antigens, and whatever differences there were between the individual strains were not sufficiently marked or consistent to justify a classification on this basis. Any differences in titer of the individual antigens seemed to be due to the efficiency or lack of efficiency of the individual antigens rather than to any specific relationship between serum and antigens. Certain antigens gave a uniformly high titer with all serums, while certain others were uniformly low. A comparison with a meningococcus antigen showed that the titer was in no case so high as with the gonococcus antigens, though the reaction was positive in low dilutions. Normal serums gave negative reactions with all antigens.

The agglutination reaction was also used as a basis of an attempted typing of strains.

Serums immune to 8 individual strains were tested against the eight antigens. Antigen was prepared by washing off a 48-hour culture in salt solution containing 0.4% formalin, centrifugalizing the suspension and resuspending the sediment in formalinized salt solution. The tendency of cultures to autolyze required that the growth to be used for an agglutinating antigen should be taken from fairly dry medium

with no water of condensation. The period of agglutination was 2 hours at 56 C. and overnight in the icebox. The average agglutination titer of the serums was from 1:600 to 1:1200.

The results of these tests gave no further basis of typing than did the fixation reactions. No groups were obtained. With these tests, also, the specific strain often gave a less marked reaction than the heterologous strains. With several serums a slightly positive reaction was obtained with meningococcus. The reactions were in no case so marked as with the gonococcus, however.

Whether the lack of typing obtained by the fixation and agglutination reactions was due to the inadequacy of these tests for this purpose or to a real similarity of the strains was not demonstrated. Further work on the question of relationship between the individual strains was therefore undertaken, using the method of absorption of agglutinins —a procedure by means of which Torrey [35] established his 3 groups and according to which Hermanies [16] has recently defined 6 groups.

The technic was essentially that given by Hermanies. The dilution of serum was determined, from which all agglutinins were absorbed by the specific strain. Having established this, a somewhat higher dilution such as 1:40 instead of 1:20, for instance, was used for absorption by the heterologous strains. The antigens used for absorption were the washed sediment from the growth of four to six testicular agar slants. Antigen for absorption was always used in excess. Serums were absorbed at 56 C. for from 6 to 12 hours and placed in the icebox overnight. On the following morning, the serums were centrifugalized and the clear supernatant fluid used for the test with the immunizing strain of the serums as the antigen. Table 6 indicates the results of absorption tests on seven serums.

It appears from the foregoing experiments that there is no evidence of grouping among the 16 strains of gonococci used. It would seem from the absorption of serum G 1, that strains G 1, G 3, G 4, G 5, G 6, G 11, G 12, G 14, G 15, and G 16 are of one group, as they all remove the major agglutinin from the serum immunized to G 1. It would then be expected that if a serum immune to any one of these strains should be absorbed with the 16 strains that identical results would be obtained. Rabbits were therefore immunized to strains G 11 and G 16. From the absorption of serum G 11, we find that strains G 7, G 9, G 10, and G 11 remove the major agglutinins and from the

[35] Jour. Med. Res., 1907, 16, p. 329.

absorption of serum G 16 that strains G 3, G 9, G 10, G 14, and G 16 remove the major agglutinins. It can be seen that the results are not identical: many of the strains which removed the agglutinins from serum G 1 have failed to remove the agglutinins from serums G 11 and G 16; strains also removed the agglutinins from the latter serums which failed to remove the agglutinins from the former serum.

Two rabbits were immunized against strain G 8. The absorption experiments with these serums gave dissimilar results, as shown in table 6. Other serums were absorbed, but the agglutinins were removed with no uniformity.

The present work has added nothing to the question of typing of the gonococcus. Our results with fixation and agglutination tests

TABLE 6
RESULTS OF ABSORPTION EXPERIMENTS

Absorbing Strains	Immune Serums						
	G 1 Serum	G 8 Serum 1	G 8 Serum 2	G 9 Serum	G 11 Serum	G 13 Serum	G 16 Serum
G 1	+	—	—	—	—	—	—
G 2	—	—	—	—	—	+	—
G 3	+	+	—	+	—	+	—
G 4	+	—	—	—	—	—	—
G 5	+	—	+	—	—	—	—
G 6	+	—	—	—	—	—	—
G 7	—	+	+	-	+	+	—
G 8	—	+	+	—	—	—	—
G 9	—	+	—	+	+	—	+
G 10	—	+	+	+	+	+	+
G 11	+	—	—	—	+	—	—
G 12	+	—	—	—	—	+	—
G 13	—	—	—	—	—	+	—
G 14	+	—	+	—	—	—	+
G 15	+	—	—	—	—	—	—
G 16	+	—	—	—	—	—	+

+ indicates that the agglutinins for the immunizing strain of the serum are removed.

on unabsorbed immune serums agree with those of Kolmer and Brown,[28] Wollstein,[36] and Thomsen and Vollmond,[37] none of whom obtained an absolutely clear-cut differentiation between gonococcus and meningococcus, nor a satisfactory grouping of gonococcus strains. On the other hand, Torrey,[35] Watabiki,[27] Pearce,[38] and Jötten [39] found differences between strains by means of agglutination and fixation reactions. In regard to the tests on absorbed serums, our results differ from those of Torrey [35] and Hermanies.[16] We have not been able to define any groups among our 16 strains of gonococci, absorption of agglutinins having taken place without uniformity.

[36] Jour. Exper. Med., 1907, 9, p. 588.
[37] Compt. rend. Soc. de Biol., 1921, 84, p. 326.
[38] Jour. Exper. Med., 1915, 21, p. 289.
[39] Ztschr. f. Hyg. u. Infectionskrankh., 1921, 92, p. 9.

CONCLUSIONS

Gonococcus stock cultures were found to grow satisfactorily for all routine work on testicular agar. Chocolate blood testicular agar was found to be a useful medium for increasing the vitality of a weakly growing culture. Environmental requirements of the organism included moisture of the atmosphere but not a reduced oxygen tension.

Isolation of cultures from acute cases of anterior urethritis in men was most successfully accomplished on chocolate blood testicular agar. No pure cultures of gonococci were isolated from chronic cases of gonorrheal endocervicitis, although single colonies of organisms morphologically typical gonococci were obtained on plates of hydrocele testicular agar containing certain members of the triphenylmethane series of dyes as an inhibitor of contaminating organisms.

The alexin fixation test serves as an aid in diagnosis, but it should be still considered rather as confirmatory evidence than as an independent basis of diagnosis. It is of little value in early cases, as might be expected. A nonspecific reaction was obtained on the intracutaneous injection of a preparation of gonococci. A like reaction was obtained in gonorrheal patients on the injection of a preparation of meningococci.

No typing of strains of gonococcus was obtained by means of the alexin fixation and agglutination reactions or by means of the method of absorption of agglutinins.